REMARKS

The Specification has been objected to and the claims rejected under 35 U.S.C. § 112, first paragraph, the Examiner asserting that insufficient guidance is provided for a skilled artisan to recombinantly express in vivo a gene encoding an enzyme, cytokine, etc. for therapeutic use at a therapeutic level with proper regulation of expression. Applicants respectfully traverse.

Applicants' claimed invention is a specific delivery device and related uses thereof, as reflected by the recited elements in the claims (e.g., "A device for implanting autologous vascular smooth muscle cells transduced with a gene of interest in a patient..." [claim 1; emphasis added]) and the specific teachings provided in the Specification. Applicants do not purport to be claiming or teaching fundamentals aspects of gene therapy itself.

The scientific and patent literature provides an artisan with a wide variety of teachings for implementing specific gene therapy protocols, e.g., selecting a gene of interest, appropriate vectors and control elements, etc. By virtue of this body of knowledge, many clinical trials have been approved by the FDA and the NIH's Recombinant Advisory Committee ("RAC").

For example, even though the Examiner refers to an editorial by Anderson (Human Gene Ther. 5:281-282 (May 1994)) as evidence for the alleged unpredictable nature of recombinant delivery of therapeutic genes, Anderson had also provided a Declaration in the file of U.S. Patent No.

5,399,346 (issued to Anderson et al.) that emphasized the general predictability of gene therapy:

> "2. He [Anderson] has attached hereto as Exhibit 1 a list of human gene therapy protocols, and to the best of his information and belief, the protocols listed as 1-38 have been approved by the Recombinant DNA Advisory Committee (RAC), a committee of the National Institutes of Health....
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> 4. The approved protocols 5-38 are directed to human gene therapy with a variety of DNA sequences, employing a variety of delivery vehicles, and are directed to both ex vivo and in situ (in vivo) transduction of human cells. Thus, for example, such protocols include the following:
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> 1. TNF, which is a secreted cytokine
> 2. IL-2, a secreted lymphokine;
> 3. LDL receptor, a membrane protein;
> 4. TK, an activatable viral gene;
> 5. HLA-B7, a cell surface antigen;
> 6. HIV-gp120, a surface antigen;
> 7. IL-4, a cytokine;
> 8. antisense-RAS, an antisense molecule to an oncogene;
> 9. p53, a tumor suppressor gene;
> 10. CF, an integral membrane transport protein;
> 11. GM-CSF, a hematopoietic colony stimulating factor;
> 12. gamma interferon, a cytokine;
> 13. MDR, a membrane transport protein;
> 14. glucocerebrosidase, an intracellular enzyme;
> 15. mutated HIV, a viral protein;
> 16. Rev, a viral transcription factor;
> 17. anti-IGF-1, an antisense molecule to a cell growth factor; and
> 18. ribozyme, an RNA-cleaving RNA molecule.
>
> In addition, **the RAC-approved protocols encompass a wide variety of delivery means, such as retroviral vectors, adenovirus vectors, liposomes for delivery of plasmid DNA, and viral-producer cells**....
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> 5. To the best of his information and belief, **the RAC does not approve a human gene therapy protocol unless there is a reasonable expectation of efficacy**....[I]t was now possible for those skilled

> in the art to design and obtain RAC approval for a wide variety of human gene therapy protocols. [Declaration of W. French Anderson, M.D., dated October 15, 1993, in file of US Pat. No. 5,399,346; emphasis added.]

A copy of the Anderson Declaration is provided herewith for the Examiner's convenience as Exhibit 1.

As can be seen from the above, examples of each of the gene products enumerated by the Examiner (enzymes, cytokines, receptors, hormones, growth factors, and coagulation factors) have been accepted for clinical trials for human gene therapy. And, as stated by Anderson, "the RAC does not approve a human gene therapy protocol unless there is a reasonable expectation of efficacy." A patent Specification need not teach, and preferably omits, that which is already known in the art. While Applicants provide guidance for use of gene therapy protocols in the context of their claimed invention, i.e., "a device for implanting autologous vascular smooth muscle cells transduced with a gene of interest," Applicants are not required to teach and enable all forms of gene therapy. To the contrary, Applicants provide a novel and nonobvious device for implementing the delivery of genes which are developed in accordance with the general teachings of the art.

The Examiner points to claim 19 as justifying the position taken in the Office Action regarding the objection to the Specification. Claim 19 recites "engrafting a device as in claim 1 into the patient, wherein the transduced cells constitutively express an insulin or proinsulin polypeptide." The Examiner is concerned, apparently, that the guidance provided in the Specification does not identify "appropriate target cells" to achieve a level of expression necessary to treat the disease. The target cells are clearly identified in claim 1 (from which 19 depends) however, as "autologous smooth

muscle cells." As far as levels of expression required to treat the disease, insulin levels sufficient to have a therapeutic effect would be evident to the clinician monitoring such a patient, and are readily (and routinely) monitored in diabetic patients. While disease due to insufficient or defective insulin receptor may or may not be treated by the device and related methods, Applicants do not purport to cover all possible treatment protocols but rather those which are suitable to treatment with Applicants' devices and methods. Applicants have amended claim 19 to better clarify this aspect.

The Examiner states that the treatment of anemia by implantation of erythropoietin-expressing cells is an analogous example of a genetic treatment dependent on tight regulation of gene expression. The basis for the Examiner's assertion is not set forth. To the contrary, it is not believed necessary to tightly regulate erythropoietin for therapeutic use. If the Examiner believes otherwise the basis for it should be provided so Applicants can properly address it. It should be noted that Applicants can, however, pre-determine the amount of erythropoietin that is secreted per cm. of graft containing transduced cells. Thus to achieve a desired hematocrit one needs only implant a device having a length necessary to achieve this result (see, e.g., McCarthy, Exhibit 5, infra). As shown in Exhibit 2 hereto (Osborne et al., Proc. Natl. Acad. Sci. USA 92: 8055-8058 (Aug. 1995), an article that includes co-inventors as co-authors, long-term therapeutic expression of erythropoietin in rats has been achieved using transduced vascular smooth muscle cells for greater than seven weeks, and subsequent data have been obtained for expression out to greater than nine months. As noted therein:

> "These data indicate a relatively efficient seeding procedure that results in a cell mass **capable of providing sustained gene delivery at**

> **therapeutically significant levels**. [p. 8057; col. 1, first para; emphasis added.]
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> ***
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> The constitutive level of Epo we achieved in this study would provide useful therapy for patients with renal failure. Although arterial seeding is not feasible in human subjects, we have recently shown in baboons that prosthetic vascular grafts can be used as a device to implant transduced cells. From the data produced in this rat model and our studies in dogs and baboons, we estimate that 10^8 transduced vascular smooth muscle cells can provide a therapeutic dose of Epo to an 80-kg patient, and **this cell number could be transplanted in a 10 cm x 4 mm prosthetic graft**.... The ability to treat these patients, and others with Epo-responsive anemias, by gene therapy would provide major clinical and economic benefits. [p. 8057, col. 2, lines 13-30; citations omitted; emphasis added.]

Thus, the Examiner's concerns regarding delivery of erythropoietin do not appear to be borne out by in vivo data obtained by or on behalf of Applicants.

The Examiner asserts that working examples described in the Specification, e.g., transduction and expression of lac Z gene and the PNP gene, would not be accepted as evidence that the claimed invention can treat a diseased patient with a reasonable expectation of success. Applicants respectfully disagree. Lac Z (encoding β-galactosidase) is entirely appropriate as a model for expressing a potentially useful therapeutic protein. Lac Z is widely used as an effective gene product to identify transduced cells histologically. Simple and specific histochemical identification of transduced cells expressing Lac Z is well established throughout gene therapy and developmental biology research. For example, Lac Z was used as a marker in the baboon study described in the instant application to locate, identify and enumerate

transduced cells in a prosthetic graft before and after removal from test animals. Moreover, Applicants' work with expression of the Lac Z gene and its use as a model for gene therapy expression was accepted and published in peer-reviewed scientific journals. See, for example, Geary et al., Human Gene Ther. 5: 1211-1216 (1994), provided herewith as Exhibit 3. The Examiner's attention is also respectfully directed to the Bell patent which he cites, wherein multiple examples of β-galactosidase are described in this field (see, e.g., col. 3, line 60 to col. 4, line 39). Moreover, Applicants respectfully note that a particular duration for expression or treatment is not set forth in the claims, so a rejection of the claims on this basis is not believed appropriate.

Regarding duration of gene expression in transduced vascular smooth muscle cells, long term therapeutic levels of erythropoietin expression have been achieved as noted above in Osborne et al., Proc. Natl. Acad. Sci. USA. These cells can be readily implanted in a vascular graft as described in the instant Specification and as proposed in Osborne, id.

In addition, granulocyte-colony stimulating factor (G-CSF) has been expressed in vascular smooth muscle cells of rats and provided therapeutic levels of the cytokine (as measured by, e.g., therapeutic increases in neutrophil counts) for at least seven weeks. Lejnieks et al., Human Gene Ther. (submitted for publication), attached hereto as Exhibit 4. Additional data in dogs, which can be supplied to the Examiner if requested, show therapeutically relevant increases in neutrophil production from implanted seeded PTFE grafts. As concluded in Lejnieks (page 11), "Thus, data is accumulating to show that vascular smooth muscle cells provide an ideal target tissue for gene therapy. These cells are readily obtained, cultured, transduced and returned to their donor. Implantation of these cells in the blood circulation suggests their use for the secretion of not only hormones but also

clotting factors for the treatment of patients with hemophilia and enzymes for treatment of lysosomal storage disorders."

On page 4 of the Office Action the Examiner suggests that different vector systems contribute to the unpredictability of using the claimed invention as well as limiting its therapeutic activity. As noted above, Applicants have provided a device for implanting transduced vascular smooth muscle cells and the corresponding methods of expressing the gene product of interest. Applicants' invention does not reside in the vectors themselves. A wide variety of vectors are known in the art, each providing different advantages and posing different problems, as is well known by the artisan of ordinary skill.

Vectors which are suitable for transducing vascular smooth muscle cells are appropriate for use in the present invention. As noted in the Specification, usually these vectors will be retroviral vectors. The vascular smooth muscle cells are readily transduced with retroviral vectors, thereby providing a population of cells containing stable integrants that provide gene expression for the life time of the cells and their progeny. The vast majority of cDNA's expressing therapeutic proteins can be accommodated in retroviral vectors, including, e.g., the cystic fibrosis transmembrane conductance regulator at 4 kb. Larger genes, e.g., greater than 8 kb, can be accommodated in other vectors, e.g., the foamy virus group of retroviruses, as is described in the literature. Thus, contrary to the assertion in the Office Action, the expression of larger genes is certainly feasible.

The Examiner also refers to an article by Jolly for the general proposition that recombinant delivery of therapeutic genes is allegedly unpredictable, and more specifically regarding the design of tissue specific promoters

to be used in such vectors. As discussed above, Applicants' invention resides in the device and related methods for implanting transduced vascular smooth muscle cells in a host, not in the vectors themselves. Moreover, Jolly merely states the obvious, i.e., that some constructs have lower titers than others. In any event, the art regarded the use of such vectors sufficiently predictable to justify the 38 gene therapy trials described in the Anderson Declaration, where it was further stated that the trials would not have been approved by the RAC unless its members were convinced of "a reasonable expectation of efficacy." Regarding the concerns about different tissue-specific promoters, attention is respectfully directed to the recitation in Applicants' claims that the transduced cells are "vascular smooth muscle cells." Thus, the promoter problems encountered by Jolly for a variety of tissues is not pertinent in the present case.

The quote from Anderson (Human Gene Ther.) to the effect that many viral promoters are shut off in primary cells in vivo is not believed to be pertinent to the vascular smooth muscle cells employed in the present invention. It has been determined that this cell type does not inactivate retroviral expression sequences. See, for example, page 11 of the Lejnieks et al. manuscript (Exhibit 4), where it is stated that:

> "Our data show that transduced vascular smooth muscle cells do not inactivate retroviral vector sequences, in agreement with previous studies of retrovirally-mediated gene expression in these target cells (Lynch et al., 1992; Clowes et al., 1994; Osborne et al., 1995). This is in contrast to skin fibroblasts where vector inactivation has been documented in both rats (Palmer et al. 1991) and dogs (Ramesh et al., 1993). Thus, data is accumulating to show that vascular smooth muscle cells provide an ideal target tissue for gene therapy.

The Examiner also points to a review article by Ledley published in 1991 which addresses concerns regarding animal experiments and whether they predict efficacy in humans. While this might have been a concern in 1991, the Anderson Declaration (Exhibit 1) established that, by October 1993, 38 human clinical trials were approved by the FDA and the RAC of the NIH, each undoubtedly based at least in part on initial work in animals. The animal models employed by Applicants are accepted by workers in the field, and this is further evidenced by acceptance of Applicants' work in peer-reviewed scientific publications. Thus, the working examples described in the Specification, as well as later work by Applicants in other models, clearly support the teachings of Applicants' Specification.

On page 7 of the Office Action the Examiner comments that grafts have been shown to be susceptible to atherosclerosis and occlusion over time in vivo, and he refers to articles by Mann et al. and Stanley et al. Mann reports that "to date, there has been no successful development of such a graft." Apparently Mann was not aware of Applicants' publications, because Applicants have reported the lack of thrombogenesis as a benefit of their seeding protocol. Moreover, as noted above Applicants have data which show grafts that are patent at three months, and it is generally regarded by workers in the field that grafts that are patent at three months should remain open indefinitely because thrombosis is a relatively acute response in synthetic grafts. In any event, even if the patency period is shorter, no time limit is expressed in the claims, and the Specification expressly contemplates that grafts may need to be replaced periodically. See, e.g., page 11, line 37 ff ("If expression of the gene product diminishes over a period of time, the graft can be removed and replaced with a freshly seeded graft). Note also that in Example I the grafts remained patent during the study: "animals remained healthy and all

grafts were patent throughout the period of study." Page 16, lines 31-32. The Examiner's comment that "Applicant provides no evidence of long-term patency of the invention as claimed" is not understood, as "long-term patency" is not believed to be recited in Applicants' claims.

Regarding selection of appropriate graft material, this issue is discussed in the Specification at, e.g., page 3, line 38 ff ("the graft is conveniently a synthetic vascular prostheses of a porous flexible material that is elongate and tubular in design and suitable for use as a vascular graft"), page 4, lines 11-18 ("Typically the porous synthetic material used to form the basis of the graft is polytetrafluoroethylene (PTFE), Dacron, polyurethane, Corethane®, nylon or various composites thereof. The pores of the graft will usually be about 60 to 90 microns in diameter, and the graft material itself may be wrapped or unwrapped. The internal diameter of the graft will be of a size appropriate to the vessel in which it is to be implanted") and elsewhere throughout the Specification. The comments of Stanley are not believed to be inconsistent with Applicants' teachings regarding graft selection.

For the foregoing reasons withdrawal of the objection to the Specification and rejection of the claims under 35 U.S.C. § 112, first paragraph is respectfully requested.

Claims 1-4, 8-11, 13 and 16-20 stand rejected under 35 U.S.C. § 103 based on Noishiki et al. (US 5,387,236) in view of Nabel et al. (US 5,238,470). Noishiki is cited as disclosing a vascular prosthesis in which autologous endothelial and smooth muscle cells are deposited and captured within the walls of the prosthesis. Nabel is relied on for teaching the <u>in situ</u> transduction of endothelial and smooth muscle cells of an arterial wall, or the deposition of cells

transduced ex vivo, using a catheter to deposit the cells or other gene transfer vehicle. The Examiner states that it would have been obvious to use a synthetic vascular graft to transplant endothelial and smooth muscle cells as disclosed by Noishiki and to substitute genetically modified cells based on the teaching of Nabel to genetically modify cells of the arterial wall for therapeutic purposes. Applicants respectfully disagree with the assumptions made by the Examiner in formulating this basis for rejection and thus respectfully traverse.

The basis for the rejection does not set forth a motivation for combining the teachings of Nabel and Noishiki to reach Applicants' claimed device and methods. Nor is a motivation apparent from the references as well. The devices and methods of Noishiki are vascular grafts coated with endothelial cells, designed for surgical implantation in a patient. In contrast, the gene delivery method of Nabel involves catheterization to deliver cells or vectors to specific arterial sites. To deliver the cells (or vector) Nabel teaches that the selected region of the blood vessel is denuded of endothelium by mechanical trauma in combination with a proteolytic enzyme (e.g., col. 7, lines 22-25). Thus, the coating of endothelial cells in Noishiki is that which is specifically removed in Nabel. The teachings of the two patents, considered as a whole, cannot be combined to reach the claimed invention without resorting to selecting specific elements from each and combining them, in the absence of any motivation from the references to do so.

Even were Noishiki and Nabel properly combinable, the artisan would, perhaps, be taught to attempt to transduce in situ the cells of the Noishiki device by the process of Nabel. In contrast, Applicants' claimed device and methods recite "a device for implanting autologous vascular smooth muscle cells transduced with a gene of interest..." (claim 1).

Thus, transduction of autologous cells is performed outside of the intended recipient. See also Applicants' claim 20 ("...engrafting the device having the immobilized transduced smooth muscle cells and endothelial cells into the vasculature..."). The claims clearly distinguish over the teachings of Noishiki and Nabel, whether properly combinable or not.

As stated in In re Clinton, 188 USPQ 365, 367 (CCPA 1976), in determining obviousness or nonobviousness one first determines whether the references contain a sufficient motivation for combining them in the manner suggested by the Examiner. Applicants respectfully submit that the requisite motivation for combining the references to reach the claimed invention is absent. While the references themselves need not expressly suggest the invention, they still must collectively suggest it. In the present case, nothing has been pointed to which provides the collective suggestion of the claimed invention, or which provides a motivation for combining the references to bridge the gap that separates the references from Applicants' claimed invention. In a biotechnology related case the Board of Patent Appeals and Interferences has stated that:

> Before obviousness may be established, the examiner must show that there is either a suggestion in the art to produce the claimed invention or a compelling motivation based on sound scientific principles. See Carl Schenk A.G. v. The Norton Corp., 713 F.2d 782, 218 USPQ 698, 702 (Fed. Cir. 1983). [underscore added]

Ex parte Kranz, 19 USPQ2d 1216, 1218 (Bd. Pat. App. & Int. 1990). That Noishiki and Nabel might possibly be combined in a manner suggested by the Examiner does not provide the motivation to do so, much less the "compelling motivation" required in Kranz. And, as stated in In re Gordon, 221 USPQ 1125, 1127 (Fed. Cir. 1984):

> The mere fact that the prior art could be so modified would not have made the modification obvious unless the prior art suggested the desirability of the modification.

Gordon relies on In re Imperato, 179 USPQ 730 (CCPA 1973), a clear example of a case when the combination of the teachings of the prior art references did not suggest the inventor's result. As summarized in In re Sernaker, 217 USPQ 1, 6-7 (Fed. Cir. 1983):

> The lesson of [In re Imperato, 179 USPQ 730 (CCPA 1973)] appears to be that prior art references in combination do not make an invention obvious unless something in the prior art references would suggest the advantage to be derived from combining their teachings. [Underscore added.]

Applicants respectfully urge that nothing in Noishiki or Nabel suggests the desirability of Applicants' presently claimed combination of features. Indeed, it is doubtful that the art could have been combined in the manner suggested by the Examiner to reach the present invention.

Further, there is no suggestion in the cited art of the problems which Applicants have addressed and the advantages provided by their claimed device and methods. For example, by quantifying the number of transduced cells and tailoring the size of the device, a precise amount of gene product can be delivered to patients, a feature of importance in many medical therapies. See, for example, results described by W. Osborne in McCarthy, "Reason for Optimism Over Vascular Gene Therapy," The Lancet 347:752 (1996), attached hereto as Exhibit 5. Thus, clinicians are provided with the ability to more precisely regulate the amount of gene product as may be necessary for a particular patient or disease. Moreover, the device of the inventors also provides a long term solution to gene delivery, whereas Nabel provides a

shorter term approach, as noted by McCarthy ("But research is also under way to develop longer-term applications with vascular gene transfer. William Osborne and his collaborators [...] have developed a technique to impregnate a porous synthetic vascular graft with gene-altered cells....")

Another advantage provided by Applicants' invention, addressing a problem not raised in the combined teachings of the cited references, is that of controlled vector delivery. For example, with the instillation of transduced cells or vector according to Nabel's teachings, cells or vector which do not implant at the denuded site during the catheterization procedure are free to migrate beyond the area to other parts of the body, having unknown effect for an unknown duration. Thus, although not contemplated by either Nabel or Noishiki, complete removal of transduced cells from the patient of Nabel's procedure (as might be desired if adverse effects were encountered with the gene product) would be a difficult if not impossible task. In contrast, Applicants' devices and procedures permit convenient and periodic replenishment, or even complete replacement, of the cells which express the gene. This solves the problem that might be encountered when expression is limited in duration or amount, and addresses the situation that may necessitate removal of the genetically modified cells.

Thus, as the combination of Noishiki and Nable does not suggest Applicants' claimed devices and methods, and does not even suggest problems which Applicants' devices and methods address, withdrawal of this basis of rejection is respectfully requested.

Claims 5-7 and 12-15 stand rejected over the combination of Noishiki in view of Nabel and further in view of Anderson et al. WO 90/224,525. Anderson is cited as disclosing a vascular graft coated with modified endothelial

cells to deliver a variety of gene products. The rejected claims 5-7 depend directly or indirectly from claim 1 and are directed to smooth muscle cells transduced with specific genes, i.e., the gene encoding erythropoietin (claims 5 and 14), G-CSF or GM-CSF (claims 6 and 12) and Factor IX (claim 7), or engrafting the device into a patient's arterial system (claims 13 and 15). Anderson does not supply any of the motivation or teaching that is missing from the combination of Noishiki and Nabel. There is no suggestion from their combined teachings of engrafting a device containing autologous vascular smooth muscle cells transduced with a gene encoding a specific protein of interest into a patient's vascular system, as recited in Applicants' claims. Thus, withdrawal of this basis for rejection is respectfully requested.

Claims 21 and 22 stand rejected under 35 U.S.C. § 103 over the combination of Noishiki in view of Nabel and further in view of Bell et al. (US Patent 5,336,615). Bell is asserted to disclose engineered endothelial cells that are propagated in autologous serum before seeding denuded vessel segments of natural or synthetic grafts. Applicants respectfully disagree. The cultivation of endothelial cells is taught to be in "tissue culture or other suitable medium" (col. 7, lines 33-36). There is absolutely no suggestion of cultivation in autologous serum, to which Applicants' claims 21 and 22 are directed ("a medium containing autologous serum"). The bovine cells and fetal calf serum medium pointed to by the Examiner is not said to be autologous (from the same patient), and are most assuredly allogeneic in the absence of any teaching to the contrary. Consequently, withdrawal of this basis of rejection is requested.

In view of the above amendments to the claims and accompanying remarks, Applicants believe that each rejection has been addressed and overcome and that the application is

now in condition for allowance. Early notice to that effect is earnestly solicited.

If for any reason, however, the Examiner feels that a telephone conference would expedite prosecution of the subject application, the Examiner is invited to telephone the undersigned at 206/467-9600.

Respectfully submitted,

Dated April 2, 1996

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Encl.

Exh. 1 (Anderson Declaration from file of US 5,399,346)
Exh. 2 (Osborne et al., PNAS 92: 8055-8058 (1995))
Exh. 3 (Geary et al., Human Gene Ther. 5:1211-1216 (1994))
Exh. 4 (Lejnieks et al., Human Gene Ther. (submitted)
Exh. 5 (McCarthy, The Lancet 347:752 (1996))

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